

USSN 09/679,705

In the Claims:

Please cancel claims 1-28 and 49 without prejudice; amend the remaining claims as set forth below and add new claims 57-63.

1. (Cancelled) A prokaryotic cell that is genetically modified to shift the redox status of the cytoplasm to a more oxidative state, and which further contains a gene encoding a catalyst of disulfide bond formation and/or isomerization.
2. (Cancelled) The prokaryotic cell of claim 1, wherein the expression or activity of a reductase is decreased relative to that in the corresponding wild type cell.
3. (Cancelled) The prokaryotic cell of claim 2, wherein the reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase, and glutathione.
4. (Cancelled) The prokaryotic cell of claim 3, in which the expression or activity of a second reductase is decreased relative to that in the corresponding wild type cell.
5. (Cancelled) The prokaryotic cell of claim 4, wherein the second reductase is selected from the group consisting of thioredoxin reductase, glutathione reductase, and glutathione.
6. (Cancelled) The prokaryotic cell of claim 2, wherein the gene encoding the reductase is mutated.
7. (Cancelled) The prokaryotic cell of claim 6, wherein the gene encoding the reductase contains a null mutation.
8. (Cancelled) The prokaryotic cell of claim 5, wherein the genes encoding the first and the second reductases contain a null mutation.
9. (Cancelled) The prokaryotic cell of claim 2, wherein the activity of the reductase is inhibited.
10. (Cancelled) The prokaryotic cell of claim 9, wherein the activity of the reductase is inhibited by contacting the prokaryotic cell with an agent.
11. (Cancelled) The prokaryotic cell of claim 1, further modified to increase its ability to proliferate.
12. (Cancelled) The prokaryotic cell of claim 4, further modified to increase its ability to proliferate.
13. (Cancelled) The prokaryotic cell of claim 11, wherein the modification consists of the introduction of a suppressor mutation.
14. (Cancelled) The prokaryotic cell of claim 12, wherein the modification consists of the introduction of a suppressor mutation.
15. (Cancelled) The prokaryotic cell of claim 11, wherein the modification restores at least some of the reducing capacity to the cytoplasm of the prokaryotic cell.

USSN 09/679,705

16. (Canceled) The prokaryotic cell of claim 11, wherein the modification is a mutation in the *ahpC* gene which reduces its peroxidase activity.
17. (Canceled) The prokaryotic cell of claim 16, wherein the mutation is located in a region containing four triplet repeats.
18. (Canceled) The prokaryotic cell of claim 17, wherein the mutated *ahpC* protein has the amino acid sequence set forth in SEQ ID NO: 24.
19. (Canceled) The prokaryotic cell of claim 1, having ATCC Designation No. PTA-938 (FA112).
20. (Canceled) The prokaryotic cell of claim 1, having ATCC Designation No. PTA-939 (FA113).
21. (Canceled) The prokaryotic cell of claim 19, further comprising a nucleic acid encoding a catalyst of disulfide bond formation or isomerization.
22. (Canceled) The prokaryotic cell of claim 20, further comprising a nucleic acid encoding a catalyst of disulfide bond formation or isomerization.
23. (Canceled) A prokaryotic cell of claim 1, further comprising a heterologous nucleic acid.
24. (Canceled) The prokaryotic cell of claim 1, which comprises a nucleic acid encoding a catalyst of disulfide bond isomerization.
25. (Canceled) The prokaryotic cell of claim 24, which comprises a nucleic acid encoding a catalyst of disulfide bond isomerization.
26. (Canceled) The prokaryotic cell of claim 25, wherein the catalyst is a DsbC protein or an analog thereof which lack a signal peptide.
27. (Canceled) The prokaryotic cell of claim 1, wherein the catalyst is a variant of a protein of the thioredoxin superfamily having a redox potential that is higher than that of its wild type counterpart.
28. (Canceled) The prokaryotic cell of claim 27, wherein the variant is a "Grx" variant of thioredoxin A.
29. (Currently amended) A prokaryotic cell that is genetically modified to shift the redox status of the cytoplasm to a more oxidative state that favors disulfide bond formation, relative to wild type a prokaryotic cell that is not genetically modified, which cell further comprises a mutated AhpC gene comprising an insertion of three nucleotides in the TCT triplet rich region located at about codons 36-39 of an AhpC gene, which insertion is further genetically modified to increases the cell's ability to proliferate relative to a cell that is not further genetically modified.

USSN 09/679,705

30. (Previously presented) The prokaryotic cell of claim 29, in which the expression or activity of a reductase is decreased relative to that in the corresponding wild-type cell.

31. (Previously presented) The prokaryotic cell of claim 30, wherein the reductase is selected from the group consisting of thioredoxin reductase and glutathione reductase.

32. (Previously presented) The prokaryotic cell of claim 30, in which the expression or activity of a second reductase is decreased relative to that in the corresponding wild-type cell.

33. (Previously presented) The prokaryotic cell of claim 29, wherein the second reductase is selected from the group consisting of thioredoxin reductase and glutathione reductase.

39. (Currently amended) The prokaryotic cell of claim 29, wherein the further genetic modification is a suppressor mutation three nucleotides are TCT.

40. (Currently amended) The prokaryotic cell of claim 29, wherein the further modification restores at least some of the reducing capacity to the cytoplasm of the prokaryotic cell relative to cell that is not further genetically modified TCT triplet rich region of the mutated AhpC gene encodes a stretch of four phenylalanines.

41. (Currently amended) The prokaryotic cell of claim 40, wherein the further modification is a mutation in the AhpC gene which reduces its peroxidase activity mutated AhpC gene encodes a protein comprising SEQ ID NO: 11.

42. (Currently amended) The prokaryotic cell of claim [[41]] 40, wherein the mutation is located in a region containing four triplet repeats TCT triplet rich region has the nucleotide sequence set forth in SEQ ID NO: 10.

43. (Currently amended) The prokaryotic cell of claim [[42]] 41, wherein the mutation results in mutated AhpC gene encodes a mutated AhpC protein that has the amino acid sequence set forth in SEQ ID NO: 24.

44. (Original) The prokaryotic cell of claim 29, further containing a gene encoding a catalyst of disulfide bond formation and/or isomerization.

45. (Previously presented) The prokaryotic cell of claim 44, wherein the catalyst is a DsbC protein which lacks a signal peptide.

46. (Currently amended) The prokaryotic cell of claim 44, wherein the catalyst is a variant of a protein of the thioredoxin superfamily having one or more mutation in the active site motif

USSN 09/679,705

CXXC (SEQ ID NO: 1) which provides the protein with a redox potential that is higher than that of its wild-type counterpart.

47. (Original) The prokaryotic cell of claim 46, wherein the variant is a "Grx" variant of thioredoxin A.

48. (Original) The prokaryotic cell of claim 44, wherein expression of the gene encoding the catalyst is inducible.

49. (Canceled) A method for producing a protein having at least one disulfide bond, comprising growing a host cell of claim 1 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein from the host cell.

50. (Currently amended) A method for producing a protein having at least one disulfide bond comprising: growing a prokaryotic cell of claim 29 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein from the host cell.

51-54. (Canceled)

55. (Previously presented) The prokaryotic cell of claim 29, having ATCC Accession No. PTA-938.

56. (Previously presented) The prokaryotic cell of claim 29, having ATCC Accession No. PTA-939.

57. (New) A method for producing a protein having at least one disulfide bond comprising: growing a prokaryotic cell of claim 39 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein.

58. (New) A method for producing a protein having at least one disulfide bond comprising: growing a prokaryotic cell of claim 40 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein.

59. (New) A method for producing a protein having at least one disulfide bond comprising: growing a prokaryotic cell of claim 41 comprising a nucleic acid encoding a protein having at

USSN 09/679,705

least one disulfide bond, under conditions in which the protein is produced, and isolating the protein.

60. (New) A method for producing a protein having at least one disulfide bond comprising: growing a prokaryotic cell of claim 42 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein.

61. (New) A method for producing a protein having at least one disulfide bond comprising: growing a prokaryotic cell of claim 43 comprising a nucleic acid encoding a protein having at least one disulfide bond, under conditions in which the protein is produced, and isolating the protein.

62. (New) The method of claim 46, wherein the protein of the thioredoxin superfamily is TrxA.

63. (New) The method of claim 62, wherein the active site motif comprises SEQ ID NO: 3, 4, 5 or 6.